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RESEARCH ARTICLE

Effect and mechanism of commonly used four nitrogen fertilizers and three organic fertilizers on *Solanum nigrum* L. hyperaccumulating Cd

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Abstract

Solanum nigrum L. is a hyperaccumulator and shows very high phytoremediation potential for Cd-contaminated soil. Fertilizer addition to soil is an effective pathway to improve Cd hyperaccumulation. This article compared the strengthening roles of commonly used four nitrogen fertilizers with three organic fertilizers on *S. nigrum* hyperaccumulating Cd at the same total nitrogen level. The results showed that Cd concentrations in roots and shoots of *S. nigrum* were not affected by the addition of inorganic nitrogen like NH_4HCO_3, NH_4Cl, $(NH_4)_2SO_4$, and CH_4N_2O compared with the control without nitrogen addition. However, Cd concentrations in *S. nigrum* roots and shoots were significantly decreased ($p < 0.05$) when the organic nitrogen was added in the form of chicken manure, pig manure, and commercial organic fertilizer (by 15.6% and 15.1%, 30.1% and 23.6%, 20.3% and 16.8%, respectively). On the other hand, of all nitrogen treatments, the addition of $(NH_4)_2SO_4$ and CH_4N_2O to the soil enormously increased *S. nigrum* biomass, i.e., *S. nigrum* shoot biomass increased 2.0- and 2.1-fold compared with the control. Correspondingly, Cd loads in *S. nigrum* shoots were also the highest in former two treatments and amounted to 79.91 μg pot^{-1} and 80.17 μg pot^{-1}, respectively. Compared with the control, the addition of three organic fertilizers significantly increased ($p < 0.05$) pH and decreased ($p < 0.05$) available Cd concentrations in the soil, which could be the main reasons for their negative effects on *S. nigrum* accumulating Cd. $(NH_4)_2SO_4$ and CH_4N_2O significantly increased *S. nigrum* biomasses and exerted no effects on the available soil Cd concentration, which made them more better fertilizers in practice. In general, the same fertilizer may show different effects on different hyperaccumulators. The selection of fertilizer should be decided in accordance with the specific conditions in the phytoremediation practice of contaminated soil.

Keywords Cadmium · Contaminated soil · Phytoremediation · Fertilizer · *Solanum nigrum* L.

Introduction

Heavy metal pollution is becoming an increasingly significant problem in the world (Liu et al. 2015). According to the survey results of the Chinese Environmental Protection Bureau (2013), nearly one-fifth of the arable land (2×10^7 ha) in China is polluted by heavy metals, which directly caused the pollution of about 1.2×10^5 tons of crops every year (He et al. 2014). Among these heavy metal pollutants, cadmium (Cd) causes more serious pollution than other pollutants (Dai and Wei 2018). Usually, physical and chemical remediation methods are effective in the remediation of heavy metal–contaminated soil. However, these methods are difficult to use in practice due to their high costs and high possibility of secondary pollution (Bhargava et al. 2012; Lan et al. 2013). Thus, phytoremediation methods for removing heavy metals from contaminated soils by hyperaccumulator showed better prospects. Although there were dozens of Cd hyperaccumulators reported (Li et al. 2017), only a limited number of them demonstrated a higher phytoremediation potential. Among them, *Sedum alfredii* Hance (Pan et al. 2016), *Solanum nigrum* L. (Wang et al. 2015), *Bidens pilosa* L. (Dai

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et al. 2017), and *Brassica juncea* L. (Chowdhary et al. 2018), have been extensively so far.

Solanum nigrum has been identified as a Cd hyperaccumulator from among 54 weed plants of 20 families by pot and plot experiments (Wei et al. 2005). In a Pb and Zn mine field, *S. nigrum* was found to accumulate from 110 to 460 mg kg^{-1} Cd in its shoots when Cd concentrations in the soil ranged from 20 to 60 mg kg^{-1} (Wang et al. 2015). Particularly, *S. nigrum* showed a particularly strong ability to remove Cd from contaminated soil in the field experiment (Niu et al. 2015). Some effective and practical methods have been explored in order to increase Cd concentration and biomass using appropriate measures to maximize phytoremediation potential. Measures aimed at increasing the concentration of hyperaccumulators involved the enhancement of CO_2 (Li et al. 2014), endophytic bacterium (Chen et al. 2015; Pan et al. 2016), some organic acids (Wei et al. 2016), plant growth–promoting bacteria (Pan et al. 2017), chelator and electric fields (Luo et al. 2018), and so on. The addition of fertilizer to soil is an effective way to increase plant biomass. Usually, the nitrogen-containing fertilizers performed better because phosphorus in polluted soil easily caused a decrease in heavy metal content in hyperaccumulators (Guo et al. 2017). The results showed that Cd extraction efficiency (μg pot^{-1}) from rice plants significantly increased with the addition of urea and ammonium sulfate ($(NH_4)_2SO_4$) (Jalloh et al. 2009). Amonium nitrate (NH_4NO_3) and $(NH_4)_2SO_4$ also increased the biomass of sweet sorghum (*Sorghum biocolor* L.) and promoted lead enrichment (Zhuang et al. 2009). Some organic fertilizers such as pig manure, cattle manure, and compost also significantly increased plant biomass, but considerably reduced availability of heavy metal concentrations in the soil (Khan et al. 2018).

Ammonium bicarbonate (NH_4HCO_3), ammonium chloride (NH_4Cl), $(NH_4)_2SO_4$, and urea (CH_4N_2O) and chicken manure (pure fermented chicken manure), pig manure (pure fermented pig manure), and commercial organic fertilizer are the four types of inorganic nitrogen fertilizers and the three types of organic nitrogen fertilizer, respectively, which are commonly used in agricultural production. Previous experiments showed that NH_4HCO_3 and NH_4Cl not only increased Cd concentrations in the shoots of *Rorippa globosa* (Turcz.) Thell., but also increased its shoot biomass (Wei et al. 2015). The $(NH_4)_2SO_4$ and CH_4N_2O increased shoot biomass but had no effect on Cd concentrations in *R. globosa* (Wei et al. 2015). Although chicken manure reduced the concentration of available Cd in the soil, it greatly increased the biomass of *S. nigrum*, and ultimately significantly improved its remediation ability (Wei et al. 2010). However, there is a question which one or two of the four common chemical nitrogen fertilizers and three organic fertilizers can more effectively promote the excessive accumulation of Cd in *S. nigrum*. We hypothesized that a chemical nitrogen fertilizer could be more efficient in promoting Cd accumulation than an organic nitrogen fertilizer, and the same fertilizer might have a different effect on various hyperaccumulators.

Materials and methods

Soil and its properties

Soil sample is meadow soil and its soil texture is middle loam soil with pH 6.94, organic material 15.18 mg kg^{-1}, total N 0.85 g kg^{-1}, available K 80.96 mg kg^{-1}, CEC 22.9 cmol kg^{-1}, clay 22.1%, silt 43.4%, and sand 34.5%, which was collected from the field of Shenyang ecological experimental station of the Chinese Academy of Sciences (41°31′ N and 123°41′ E). This soil belongs to neutral soils. The collected soil sample was very clean compared with the soil environmental quality risk control standard for soil contamination of agricultural land (SEQ 2018).

Experiment design

Eight treatments were designed and arranged according to the common fertilization amount added in agricultural production and Cd contamination status of about 2 mg kg^{-1} in the field (Huo et al. 2018). Details are listed in Table 1. The concentration of Cd treatment in the form of $CdCl_2$ $2.5H_2O$ spiked to soil was 2 mg kg^{-1}. The total amount of nitrogen added in all types of fertilizers was the same in order to make them comparable. Nitrogen fertilizers with total N concentration of 200 mg kg^{-1} were added into the soil together with Cd according to the experimental design (Table 1). After Cd and N fertilizers were added into the soils at spring, all treated soils were filled in pots (φ = 20 cm and H = 15 cm) with 2.5 kg of soil (dry weight) each, and were subsequently equilibrated for 2 months. Inorganic and organic fertilizers were all bought from local market. Cd, Pb, Cu, and Zn concentrations in these fertilizers were basically largely lower than that of in the collected soil (Table 1).

Soil pot experiments

The seeds of *S. nigrum* were collected from the field at the Shenyang ecological experimental station of the Chinese Academy of Sciences. The station is semi-moist continental climate with total annual radiation of the area of 520–544 kJ cm^{-2}, average annual precipitation of 650–700 mm, and approximately 127–164 frostless days a year (Wei et al. 2010). The seedlings were grown on a seedling tray before experiment to ensure that the size of *S. nigrum* was the same in each treatment. When all seedlings of *S. nigrum* had four leaves and the same height, six seedlings were transplanted to each pot. Each treatment was repeated three times. All pots were

Table 1 Experimental design with nitrogen fertilizers and background concentrations of heavy metals

Treatment	Nitrogenous fertilizer	Dose spiked (g kg^{-1})	Added total N (mg kg^{-1})	Background concentration (mg kg^{-1})			
				Cd	Pb	Cu	Zn
CK	Control, no N addition	0.00	0.00	0.16	16.5	15.3	39.8
T1	NH_4HCO_3	1.12	200.00	0.01	0.12	0.14	0.52
T2	NH_4Cl	0.76	200.00	0.01	0.13	0.11	0.64
T3	$(NH_4)_2SO_4$	0.94	200.00	0.02	0.14	0.11	0.76
T4	CH_4N_2O	0.43	200.00	0.01	0.11	0.12	0.51
T5	Pure fermented chicken manure	17.87	200.00	0.03	1.48	1.21	2.42
T6	Pure fermented pig manure	28.57	200.00	0.03	1.54	1.34	2.28
T7	Commercial organic fertilizer	1.6	200.00	0.04	1.63	1.17	2.15

Doses of inorganic compounds are supplemented for analytically pure reagents; organic fertilizers in addition to soil are pure product

randomly arranged under plastic cloth for shelter from rain in the Shenyang ecological experimental station of the Chinese Academy of Sciences. Tap water was replenished every day to maintain about 80% of soil water-holding capacity. All plants and their corresponding soil samples were harvested when plants reached maturity after 118 days of growth.

Sample analysis and data processing

Plant samples were divided into two parts, i.e., roots and shoots, and then rinsed with EDTA to chelate and remove Cd attached to the roots. Subsequently, they were washed with deionized water. Firstly, these samples were dried in the oven at 105 °C for 5 min, secondly, at 70 ° overnight until completely dry. Lastly, these dried plant samples were ground and sieved through a 2-mm sieve. Plants and air-dried soil samples were digested with 87% guarantee reagent HNO_3 and 13% guarantee reagent $HClO_4$ (*v*/*v*) for detection and analysis. The available Cd concentration in the soil was extracted with 1 mol L^{-1} $MgCl_2$. Heavy metal concentration was determined using an atomic absorption spectrophotometer (AAS, Hitachi 180). The wavelengths used for Cd, Pb, Cu, and Zn are 228.8, 283.3, 324.8, and 213.8 nm, respectively. The quality control sample certified reference materials were used for total Cd concentration determination, i.e., GBW10015 (GSB-6) for plant sample and GBW07405 (GSS-5) for soil sample, which have standard Cd values of 0.15 mg kg^{-1} and 0.45 mg kg^{-1} respectively. The measured values were 0.14 mg kg^{-1} and 0.46 mg kg^{-1}, which were within expected standard ranges. Common methods were used to determine organic matter, nitrogen, and extractable P, i.e., potassium dichromate method, Kjeldahl, and sodium bicarbonate extraction method (Lu 2000). pH value was determined by a pH meter (PHS-3B), in which the ratio of soil to water was 1:2.5 (*v*/*w*) (Wei et al. 2015).

The average and standard deviation (SD) of three replicates for each treatment were performed using Microsoft Excel software, and the analysis of Duncan multiple comparison was performed at $p < 0.05$ using SPSS 17.0 software (Ma 1990).

Results

Effect of different fertilizers on Cd phytoextraction by *S. nigrum*

Cd concentrations in roots and shoots of *S. nigrum* in different nitrogen treatments were listed in Table 2. Cd extraction from shoots (Cd load in shoots, μg pot^{-1}) calculated according to Cd concentration in shoots and shoot biomass was listed in Table 2 either. Compared with the control without N addition, Cd concentrations in the roots and shoots of *S. nigrum* did not change ($p < 0.05$). However, Cd concentrations in the roots and shoots of *S. nigrum* were significantly decreased ($p <$

Table 2 Effects of different fertilizers on Cd phytoextraction by *S. nigrum*

Treatments	Roots (mg kg^{-1})	Shoots (mg kg^{-1})	Shoot extraction (μg pot^{-1})
CK	22.04 ± 1a	23.92 ± 0.51a	25.33 ± 1.77e
T1	22.14 ± 0.57a	23.93 ± 0.38a	55.76 ± 0.99c
T2	22.45 ± 0.51a	23.58 ± 0.66a	62.17 ± 3.35b
T3	23.48 ± 0.34a	25.13 ± 1.10a	79.91 ± 2.7a
T4	23.62 ± 0.11a	24.52 ± 0.47a	80.17 ± 2.16a
T5	18.6 ± 0.3b	20.32 ± 0.16b	45.78 ± 0.34d
T6	15.4 ± 0.7c	18.27 ± 0.49c	43.05 ± 1.77d
T7	17.57 ± 0.47b	19.91 ± 0.31b	45.28 ± 0.28d

Means in same columns followed by the same letter were not significantly different at $p < 0.05$

0.05) in the samples treated with organic fertilizers (chicken manure, pig manure, and commercial organic fertilizer) by 15.6% and 15.1%, 30% and 23.6%, and 20.3% and 16.8%, respectively. The highest decrease was recorded in the pig manure treatment. The change in shoot Cd extraction (μg pot^{-1}) in different treatments was not consistent with Cd concentration in *S. nigrum* shoots because Cd accumulation capacity (μg pot^{-1}) in plant shoots is a product of concentration and biomass.

Shoot Cd extractions in NH_4HCO_3 and NH_4Cl treatments were equal to 55.76 and 62.17 μg pot^{-1}, respectively, and the latter was significantly higher ($p < 0.05$) than the former. However, the same Cd shoot extraction of 79.91 and 80.17 μg pot^{-1} was obtained in $(NH_4)_2SO_4$ and CH_4N_2O treatments. Though the addition of chicken manure, pig manure, and commercial organic fertilizer similarly increased ($p < 0.05$) Cd extraction in shoots of *S. nigrum* with 45.78, 43.05, and 45.28 μg pot^{-1} compared with the control at 25.33 μg pot^{-1}, these increases ($p < 0.05$) were not that high when compared with inorganic fertilizers, indicating the huge effects of shoot biomasses.

Root and shoot biomass of *S. nigrum* in different treatments

As shown in Fig. 1, root and shoot biomasses of *S. nigrum* in the control without N addition were 0.19 and 1.06 g pot^{-1}, respectively. The addition of fertilizer significantly increased ($p < 0.05$) the biomass of *S. nigrum. Solanum nigrum* root and shoot biomasses in the treatments with chicken manure, pig manure, and commercial organic fertilizer increased 1.10 and 1.12, 1.19 and 1.19, 1.08 and 1.04 times, respectively compared with the control. However, inorganic fertilizer treatments resulted in higher biomass increases. The root and shoot biomasses of *S. nigrum* in NH_4HCO_3, NH_4Cl, $(NH_4)_2SO_4$, and CH_4N_2O treatments increased by 1.17 and 1.19, 2.24 and 1.48, 3.17 and 1.99, and 3.28 and 2.08 times, respectively, in relation to the control. Particularly, the best and similar effects were observed for $(NH_4)_2SO_4$ and CH_4N_2O ($p <$ 0.5). This result was obviously caused by different N availability in the soil between individual inorganic and organic fertilizers. Considering the effects of these fertilizers on Cd concentration in *S. nigrum* (Table 2), it is obvious that they exerted the greatest effect on plant biomasses (Fig. 1).

The pH value of the soil in different treatments

Figure 2 showed the soil pH in different treatments. After the growth of *S. nigrum*, soil pH was slightly decreased from 6.94 (original soil) to 6.85 (control) (Fig. 2). Soil pH values after *S. nigrum* growth did not changed significantly ($p < 0.05$) in four types of inorganic fertilizer treatments compared with the control (6.85). On the contrary, the addition of three types of organic fertilizers caused significant increases ($p < 0.05$) in soil pH values (by 4.28%, 4.23%, and 4.14%, respectively), which might be one of the reasons for Cd concentration decrease (Table 2).

The concentrations of extractable Cd in soils under different treatments

Figure 3 illustrates the lack of a significant increase ($p < 0.05$) of the extractable Cd in polluted soils after the addition of inorganic N fertilizers compared with the control without N addition. In contrast, organic fertilizer treatments (chicken manure, pig manure, commercial organic fertilizer) significantly descended ($p < 0.05$) the available Cd concentrations by 10.9%, 16.8%, and 12.4%, respectively, compared with the control, which was another main reason for lowered Cd concentration in roots and shoots of *S. nigrum* (Table 2).

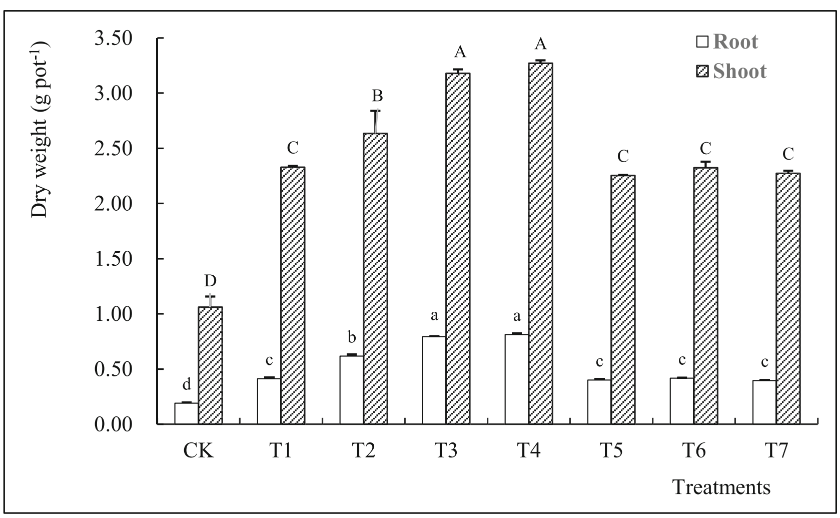

Fig. 1 Root and shoot biomass of *S. nigrum* in different treatment (means followed by different letters among treatments were significantly different at $p < 0.05$)

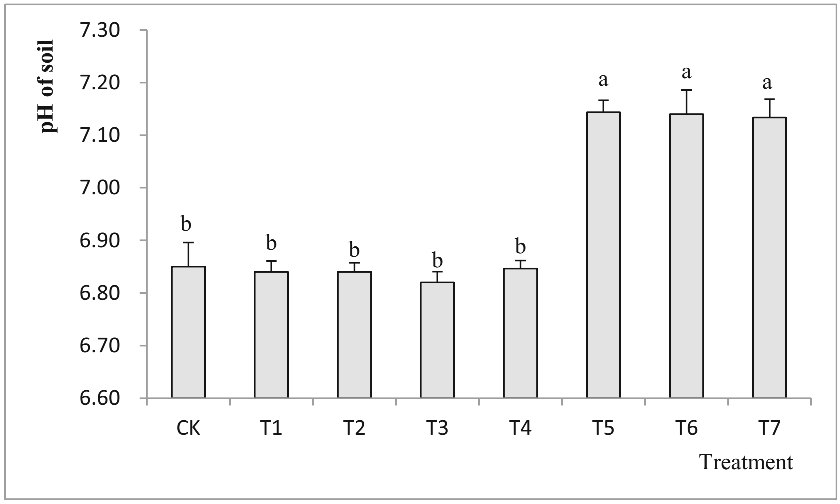

Fig. 2 The pH values of the soils in different treatments (means followed by different letters among treatments were significantly different at $p < 0.05$)

Discussion

Sedum alfredii was reported to act as a Cd hyperaccumulator. In the soil pot culture experiment which used three types of nitrogen fertilizers ($(NH_4)_2SO_4$, $Ca(NO_3)_2$, NH_4NO_3), Cd phytoextraction capacity (μg pot^{-1}) of *S. alfredii* was the highest with $(NH_4)_2SO_4$ addition due to the maximum increase of its biomass (Zhu et al. 2011). This conclusion was consistent with the results in the present experiment (Table 2, Figs. 1, 2, and 3). The effect of $Ca(NO_3)_2$ addition was very small (Zhu et al. 2011). However, another experiment concerning winter wheat accumulating Cd from soil showed that $Ca(NO_3)_2$ addition significantly increased ($p < 0.05$) Cd concentrations in the shoots and shoot biomasses (Wangstrand et al. 2007). In our previous experiment, Cd concentration in the shoots and shoot biomass of *R. globose* were significantly increased ($p < 0.05$) either (Wei et al. 2015). However, NH_4HCO_3 and NH_4Cl addition in this experiment did not improve *S. nigrum* Cd accumulation from the soil (Table 2). In fact, root exudates of different hyperaccumulators vary considerably, which has a huge impact on the concentrations of extractable heavy metals in the rhizosphere (Zhou and Song 2004). These results may suggest that the effect of the same inorganic nitrogen fertilizer on Cd accumulation may vary in different plants. Furthermore, the amount of $(NH_4)_2SO_4$ fertilizer added into the soil had a little effect on soil pH after a long period of hyperaccumulator growth due to the regulation role of root exudates (Zhou and Song 2004).

Wang et al. explored the effect of pig manure vermicompost on *S. alfredii* Cd hyperaccumulation from

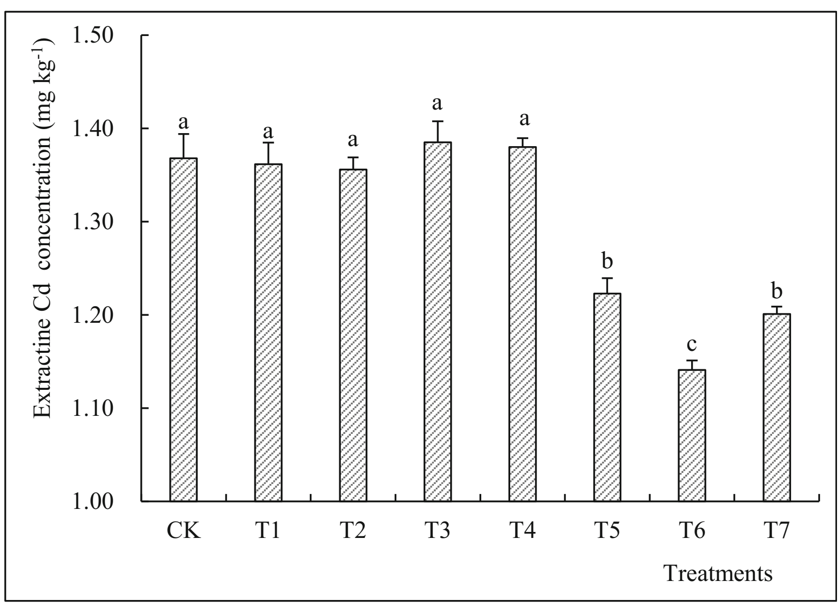

Fig. 3 The concentration of extractable Cd in different treatments in soils (means followed by different letters among treatments were significantly different at $p < 0.05$)

soil (Wang et al. 2012). The results showed that the biomass of *S. alfredii* was largely increased. Thus, the extraction of Cd (μg pot^{-1}) by *S. alfredii* was significantly increased. However, the addition of pig manure vermicompost significantly decreased Cd concentration in *S. alfredii* and available Cd concentration in the soil. These results were consistent with the experimental results of the current study. In another experiment, when composted peat was added into the soil, Cd phytoextraction (μg pot^{-1}) of *Brassica oleracea* and *Vicia faba* was significantly increased ($p < 0.05$) due to greatly increased of their biomasses, though Cd concentrations in shoots and available Cd concentrations in soils were slightly decreased (Pichtel and Bradway 2008). These results were similar to the results of this experiment (Table 2, Figs. 1 and 3).

There were many factors affecting the available Cd concentration in the soil, and pH is undoubtedly one of the most important ones (Barancíkova et al. 2004). Soil pH value and effective utilization of Cd and plant uptake are tightly associated (Tudoreanu and Phillips 2004). Within a certain range, the Cd available concentration in the soil was shown to decrease with increasing pH (Kim et al. 2009). The addition of organic nitrogen fertilizer also resulted in such trend in the present experiment (Fig. 2). In fact, the pH value of the soil polluted by heavy metals increased after adding bio-fertilizer, and the bioavailability and extraction ability (μg pot^{-1}) of heavy metals in plants decreased due to organic matter's immobilization (Houben et al. 2013). For example, when pig manure was added to the soil, the available Cd and Pb concentrations in the soil and Cd and Pb concentrations in *Helianthus annuus* were significantly decreased (Gunadasa et al. 2012), which might be the main reason why organic fertilizer reduced Cd concentration in *S. nigrum* and the available Cd concentration in the soil in this experiment (Figs. 2 and 3).

There were some field experiments concerning on *S. nigrum* phytoextraction of Cd after the addition of N fertilizers. In the plot experiment of monoculture and intercrop *S. nigrum* with low accumulation Welsh onion, the ratio of *S. nigrum* for Cd removal was about 7% in the intercrop plot when top soil (0–20 cm) Cd concentration was 0.45–0.62 mg kg^{-1} with combined N, P, and K fertilizers (2:1:1, weight ratio) added in soil before plant growth (Wang et al. 2015). In multiple crop experiment of *S. nigrum* with low accumulation Chinese cabbage, the average removal rate of *S. nigrum* to Cd was about 10% without assisted phytoextraction reagent addition for the top soil (0–20 cm) with Cd concentration at 0.53–0.97 mg kg^{-1} after its grew 90 days under the same condition of combine fertilizers of N, P, and K addition (Niu et al. 2015). Another field phytoremediation experiment was conducted in Huanjiang county of Guangxi province of China where Cd concentration in the soil was 0.71 mg kg^{-1}. The results showed that Cd concentration in *S. nigrum* was up to 16.83 mg kg^{-1}. The amount of Cd accumulated from the soil by *S. nigrum* was estimated to be more than 100 g ha^{-1} under the local normal cropping system and Cd extraction rate of more than 6%. It was assumed that only 9 years was sufficient to clean this contaminated soil. They used NaOH and organic fertilizer to regulate the acidified and Cd-contaminated soil (Zhao et al. 2015). A two-year in situ phytoremediation trial showed that Cd concentrations in the seven experimental plots with *S. nigrum* decreased from 2.75 to 1.53 mg kg^{-1}, a decrease by 24.9% after 2-year phytoremediation by *S. nigrum*, and the fertilizer application was the same as in the local agricultural production (Ji et al. 2016). Obviously, it was not enough to draw attention to the strengthening roles of nitrogen fertilizers in these experiments. Therefore, the results of this experiment provided very good reference for further field phytoremediation experiment. However, the remediation potential is still limited compared with people's expectations. Thus, we still need try to make every effort to improve its accumulation potential in the future.

Conclusion

The ability of Cd phytoextraction (μg pot^{-1}) from polluted soil by *S. nigrum* itself is limited. The addition of four types of inorganic nitrogen fertilizer (NH_4HCO_3, NH_4Cl, $(NH_4)_2SO_4$, CH_4N_2O) and three types of organic fertilizer (chicken manure, pig manure, commercial organic fertilizer) significantly increased ($p < 0.05$) Cd extracting capacity (μg pot^{-1}) of *S. nigrum*. Inorganic nitrogen fertilizer had little effect on Cd concentration in *S. nigrum* and mainly elevated Cd extraction amount (μg pot^{-1}) by greatly increasing its biomass. Organic nitrogen fertilizer significantly reduced *S. nigrum* Cd concentration, but this decrease was low. When the biomass doubled, Cd extraction (μg pot^{-1}) was eventually greatly increased. The main reason why organic nitrogen fertilizer can significantly reduce Cd concentration in *S. nigrum* may be the increased soil pH and decreased available Cd concentration in soil. The same fertilizer might play different roles in improving different hyperaccumulators accumulating the same heavy metal. In phytoremediation practice, specific problems need to be resolved and existing research results should not be simply copied.

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